Universidad Carlos III de Madrid
www.uc3m.es

University Carlos III of Madrid

Multimodal Infrared Fluorescence Endoscopy with 3D Reconstruction

Bachelor´s degree thesis

Name: Lorena Pérez Monje

Tutor: Jorge Ripoll Lorenzo

Supervisor: Pablo Gómez

Degree in Biomedical Engineering

June 2015

Acknowledgements

First, I would like to thank my parents, Gloria and Salvador, for being unconditionally supportive and helping, because without them, I would not have come this far, and especially to my father, because he did not allow me to abandon my dream at those times I had no strength. Also I would really like to thank David, my boyfriend, who has been in all those times when I felt down, listening my complains and drying my tears. To my closer friends whom have been by mi side all this time.

Finally I would like to thank all my friends at the university, especially those ones with whom I shared many days of study, practices and classes. For all their support, company and help during the degree, since without them this stage of my life would never have been the same.

Abstract

The gastrointestinal tract is one of the major sites of immunological challenges in biology. Therefore its study is very necessary for a better understanding of the biological processes that take place, in order to be used in the study of colon diseases such as colonic polyps, ulcerative colitis, diverticulitis, irritable bowel syndrome or colon cancer.

In vivo studies of small animals are key to improving human diagnosis. Despite the importance of clinical trials, they have many limitations such as in vivo imaging, which allows better understanding of biology and diseases. In vivo imaging is consider visual representation, characterization, and quantification of biological processes at the cellular and subcellular levels within intact living organisms. But not all the imaging techniques have good resolution so the research of develop better high resolution techniques enhances biological studies. so this project is trying to increase resolution and acquisition efficiency of colon imaging.

Based on in vivo imaging of small animals, the aim of this work is the image acquisition, and image processing of a multimodal infrared fluorescence endoscopy. This endoscope consists on two cameras: one of white light and the other of infrared light; that allow fluorescence imaging, giving a lot of information of the sample, such as the detection of cancer markers. A separate scanning confocal unit, that enables of cellular examinations of the colon, part of other bachelor's degree thesis of Isabel Martin Civiac.

Image acquisition is based on a motorized bed for the sample, allowing slowly control insertion of the endoscope into the sample.

With respect to image processing, the obtained images are reconstructed to give 3D images with respect the depth to offer full 3D information.

Key words: Multimodality, endoscope, fluorescence, 3D-reconstruction.

Table of Contents

Table of Figures

Table of Tables

1. Introduction

1.1 Endoscopy

An endoscopy, *Figure 1*, is a medical procedure that uses specialized tools to view and operate on the internal organs, vessels or cavities of the body. [1]

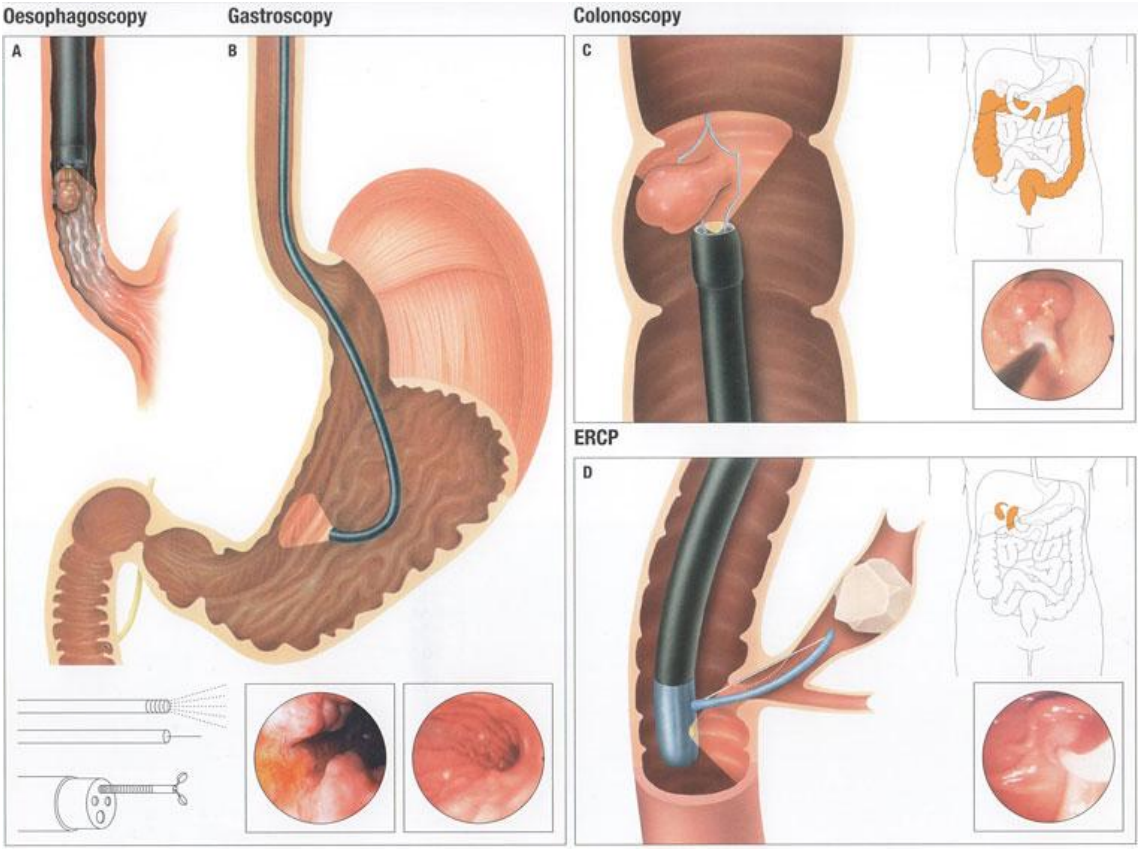

Figure 1. Different types of endoscopy [1]

The instrument used for endoscopy is the endoscope. It was developed in 1806 by Philipp Bozzini. This instrument consists on a long tube, usually flexible, *Figure 2*, useful to observe upper gastrointestinal track; but rigid endoscope are also used, *Figure 3*, in this case to penetrate tissue. It has light source and a video camera at one end, but not all the endoscopes needs a video camera since there are endoscopes in

which the images are visualized by the eyes, or transmitted to an external device. It needs a light delivery system, in order to illuminate the organ, lens system, a videoscope and in some cases an additional channel to introduce other medical instruments. The image is observed through a television screen, which allows real-time visualization; or the eye.

These kind of devices can be inserted into the body through a natural opening, such as mouth and down the throat, or the anus, or even making a small surgical cut made in the skin, called keyhole surgery. [2]

Illumination of the sample and the process of collect images are driven by the same channel.

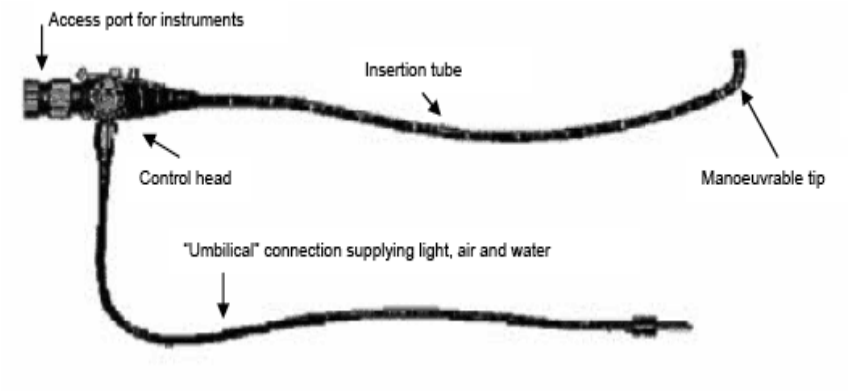

Figure 2. Flexible endoscope. [3]

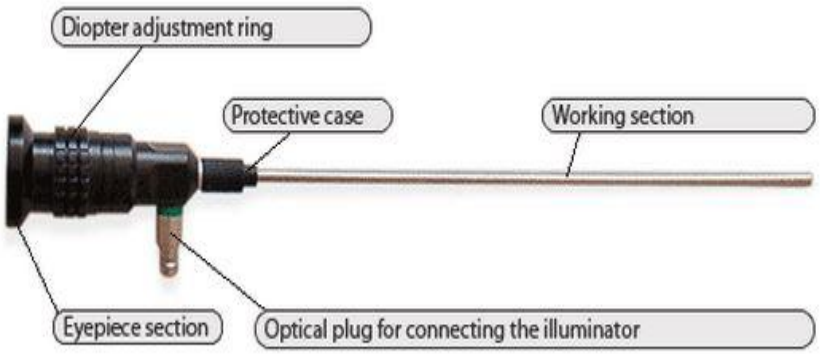

Figure 3. Rigid endoscope. [4]

There are similar devices such as borescope and fiberoscope used to visual inspection work, for example inside machines or locks.

Borescope, *Figure 4*, is an optical device that consist on a tube that can be rigid or flexible. It has an eyepiece at one end to visualize the remote object with the eye. [5]

Fiberoscope, *Figure 5*, is very similar to an endoscope consisting on a flexible fiber-optic bundle with an eyepiece on one end and lens on the other. [6]

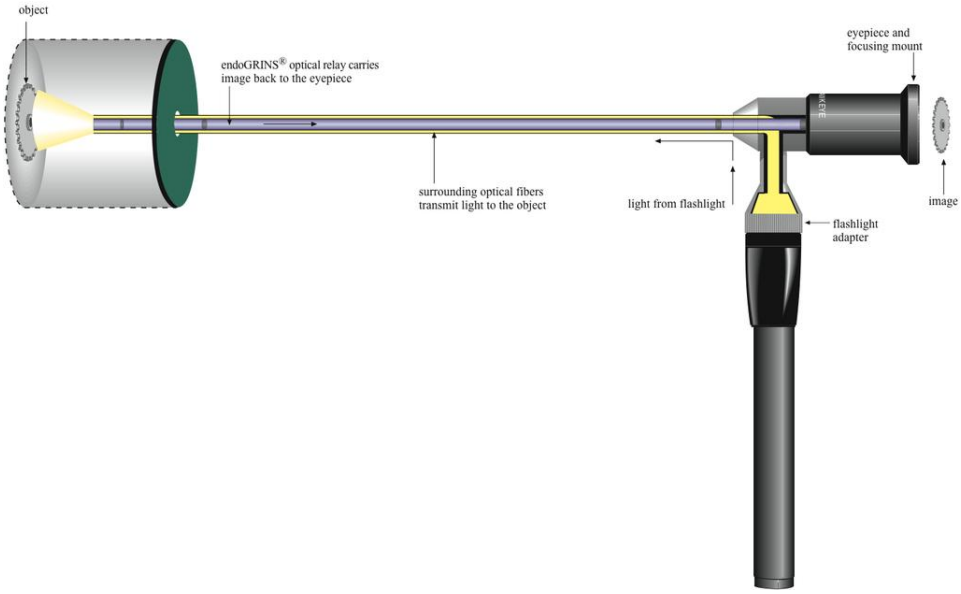

Figure 4. Borescope [5]

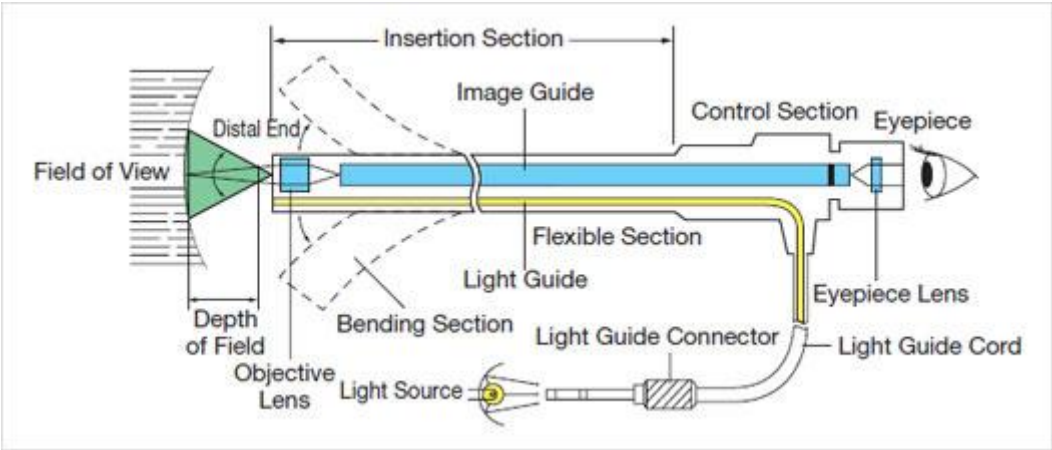

Figure 5. FIberoscope [6]

Using and endoscope, different parts of the body can be observed. Depending on the part of the body that is going to be observed, there exist different kinds of endoscopy, and also different types of endoscope since not all the cavities have the same access. The majority of endoscopes are very similar hollow tubes. *Table 1*

Name of Procedure	***Name of Tool***	***Organ***
Arthroscopy	Arthroscope	Joints
Bronchoscopy	Bronchoscope	Trachea and bronchi of the lungs
Colonoscopy	Colonoscope	Colon and Large intestine
Cystoscopy	Cystoscope	Bladder
Gastroscopy	Gastroscope	Stomach and Duodenum
Neuroendoscopy	Neuroendoscope	Brain´s areas
Laryngoscopy	Laryngoscope	Larynx

Table 1. Different kinds of endoscopies and endoscopes[7]

The most common application of endoscopy is for cancer diagnosis and other health problems, but there are other applications to treat or prevent some diseases. The endoscope can be used during surgeries or to take biopsy, but these kind of procedures require an additional channel in the endoscope, with an instrument that performs surgery, suture, cytology, or flexible forceps.

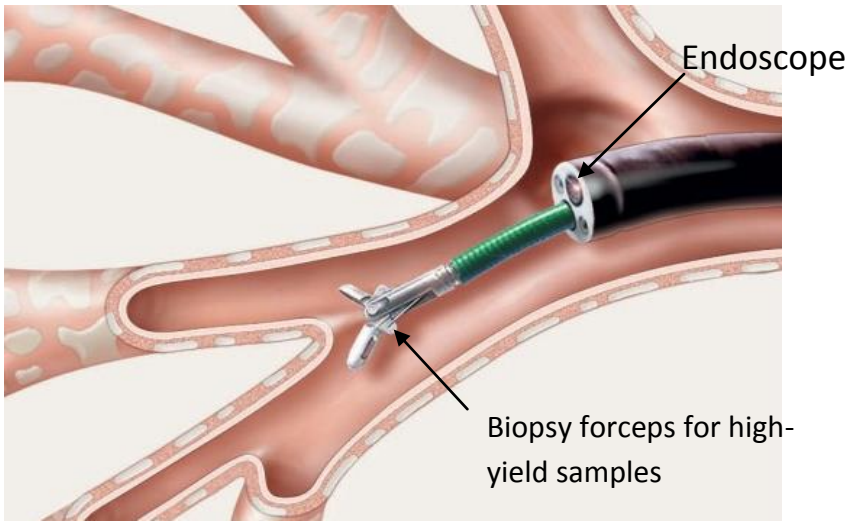

Figure 6. Bronchial Biopsy. [8]

1.1 State of the art

In recent years, some alternatives have been developed to visualize the inside of the body, and they are known as new forms of endoscopy, but with small differences with respect to conventional endoscopy.

One of the first advances is the endoscopic capsule, *Figure 5*. It arises from the need to observe the small intestine that is quite difficult because of the access. The use of an endoscopy capsule allows better observation to detect cancers, tumors or ulcers. It is used quite frequently to locate the source of bleeding, pain or other symptoms that may be located in the small intestine, and that otherwise would be difficult to locate. However, this device is not able to observe small parts as intestinal villus. [9]

The endoscopic capsule consists of an small enclosure containing a light source and a small camera. This capsule is swallowed by the patient, so that the capsule travels throughout the digestive tract, including the small intestine. During its passage, this device takes thousands of images of where it is. These images are sent to a device that the patient has located on its waist, while performing its daily activities. This occurs because the test can take between eight to ten hours. Finally the images are transferred to a computer, where they can be observed by a specialist. With respect to

the capsule, it is expelled through defecation and disposed in the toilet, so that they are single use. [9]

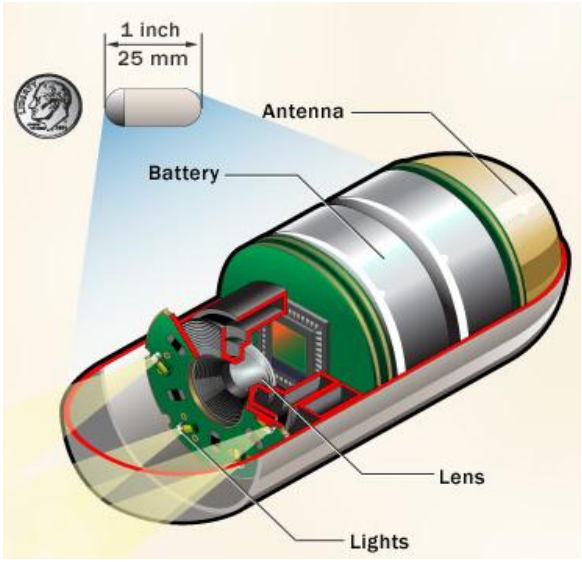

Figure 7. Endoscopic capsule [10]

Another advance in endoscopy is the virtual endoscopy, *Figure 6*, which is performed by using CT scan that computes three dimensional images of the surfaces or organs. [11]

It provides advantages over conventional endoscopy, since nothing is introduced into the body, so it is not as invasive as conventional endoscopy. It can also help too much to diagnosis, since the angle and zoom can be changed. [9]

Regarding the disadvantages, the level of detail is not as good as conventional endoscopy. In addition, remove polyps during virtual endoscopy are not allow, so the need of "normal" endoscopy should be used if abnormalities. Furthermore, the patient is exposed to a similar amount of radiation that is received in a conventional tomography. [9] [11]

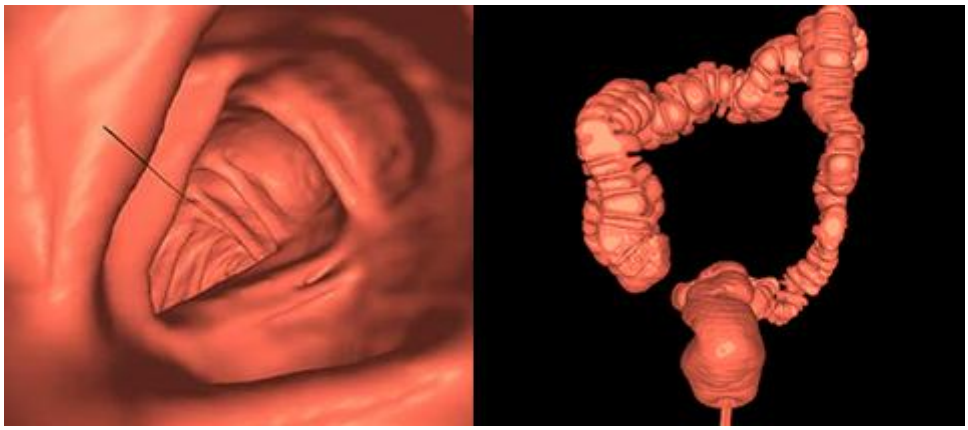

Figure 8. Virtual Endoscopic images. [12]

Some other advances are related to the combination of endoscopic fluorescence confocal microscopy and optical coherence tomography.

Including a confocal microscopy enables real-time high resolution cellular level images, so this implies a great improvement in the quality of the obtained images from a conventional endoscope. Due to the fact that it is capable of including fluorescence, allows labeling biomarkers to guide biopsies of early cancer.

A technique that combines fluorescence, confocal microscopy and endoscope, is called wide-area cellular imaging. [13]

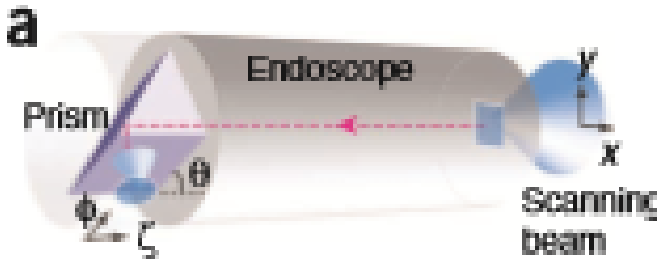

Figure 9. Schematic of a laser-scanning side-view endoscope. [13]

This endomicroscopy consist on a probe made of triplet GRIN LENS and including a right- angle prism coated with aluminum. To wide area imaging, rotational

and axial scanning is used. Rotating each frame according to the probe angle and applying image registration to display the scanned area [13]. So this kind of endoscope obtains three dimensional rendered fluorescence image. *Figure 10*

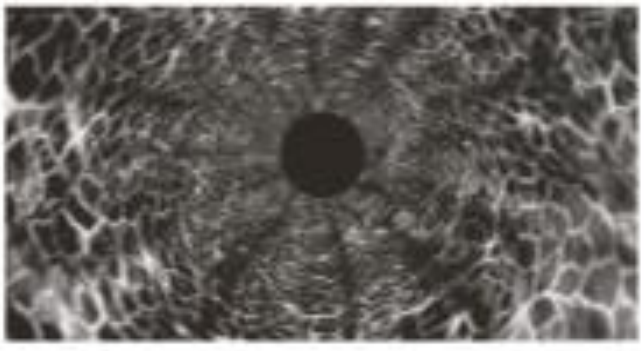

Figure 10. The vasculature in the descending colon of normal fly.

Animal models are studies that aid to understand biological phenomena. They are commonly used to research human disease when human studies are not possible.

Endoscopy for animal models is usually used, for example endoscopic OCT is used for investigation of internal organs with ultrahigh resolution, *Figure 11*.

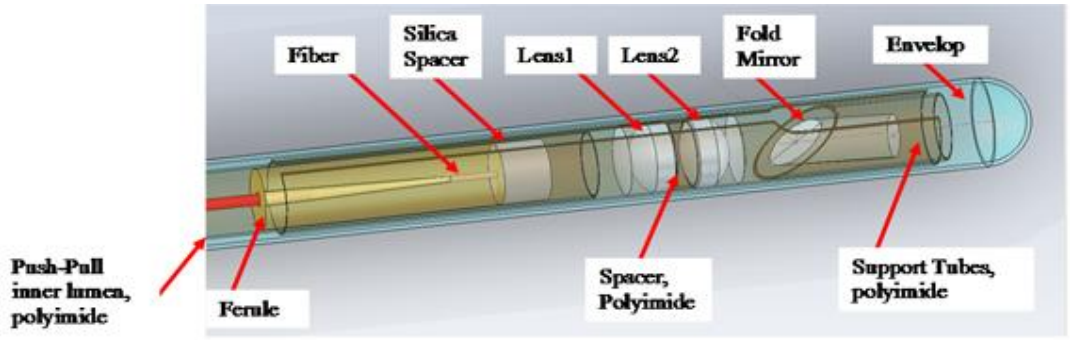

Figure 11. First in vivo ultrahigh resolution endoscopic OCT in animal model. [14]

Endoscopic optical coherence tomography is similar to ultrasonography but it employs light instead of sound waves. this technique enables micron scale, cross-sectional and three-dimensional imaging of sample microstructure in real time. [14]

Endoscopic ultrasound is a technique that combines endoscopy and ultrasounds to obtain images of the internal organs, their walls or adjacent structures in the chest, abdomen and colon. *Figure 11*

Small animal studies are useful adjunctive method for learning endoscopic ultrasound to increase the quality. [15]

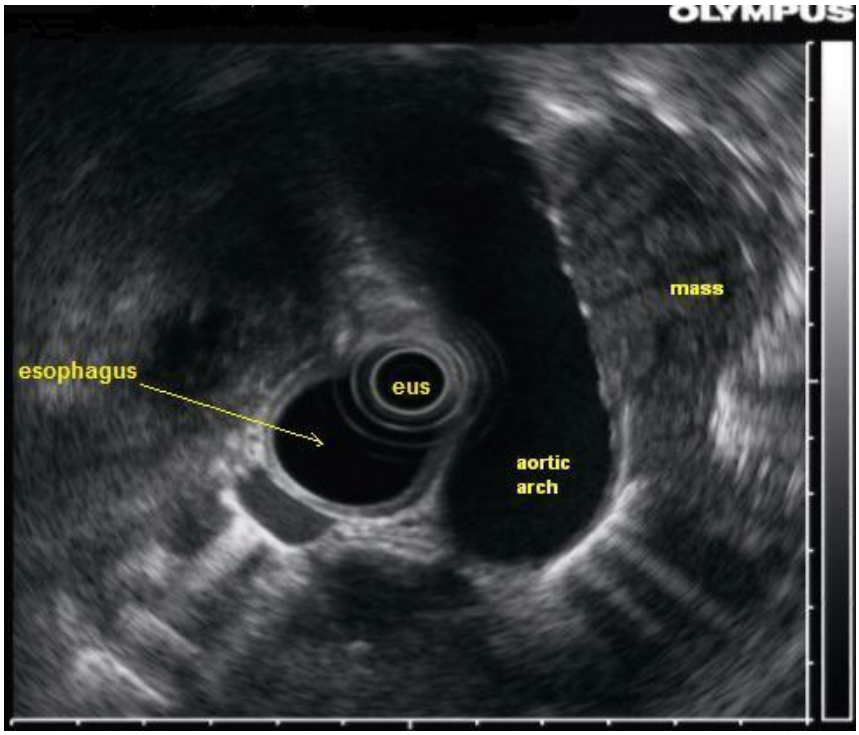

Figure 12. Endoscopic ultrasound image

2.2 Fluorescence

Fluorescence is a property of certain substances, that are able to absorb energy as electromagnetic radiation, where this radiation is transformed emitting light waves with longer wavelength than the absorbed energy. Furthermore, it is a particular type of luminescence. [16]

Generally, substances that emit light, absorb electromagnetic energy in the form of shortwave radiation, and then emit long wavelength, as previously explained.

In the case of fluorescence, the light is absorbed due to ultraviolet rays, so that the energy emitted belong to the visible region of the spectrum, ***Figure 12***. [16]

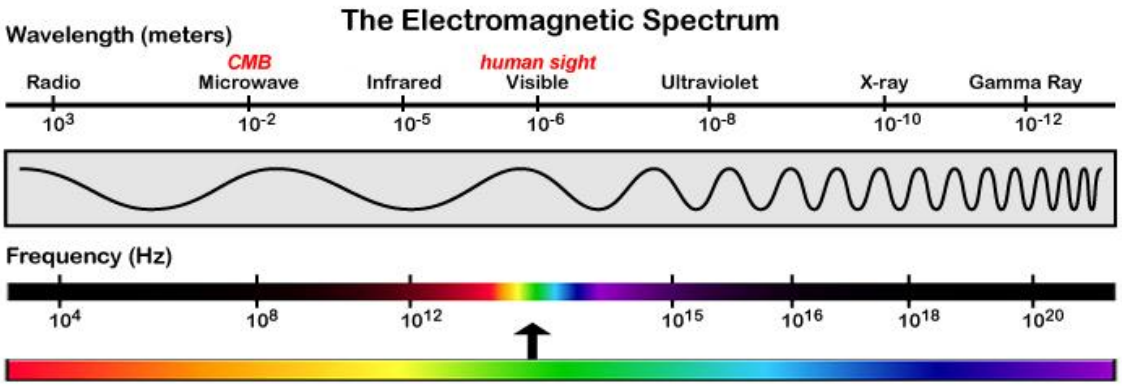

Figure 13. The Electromagnetic Spectrum [17]

Fluorescence involves three stages that occurs in special molecules that are capable of exhibiting fluorescence, called fluorophores. These stages are known as: [18]

First stage: Excitation.

Using a laser or incandescent lamp as external source, a photon is emitted with certain energy, $h\nu_{EX}$. Then fluorophore absorbs that light reaching an excited stage (S_1').

Second stage: Excited-Stage Lifetime.

The excited state lasts finite time (1-10 nanoseconds). During this time, the fluorophore suffers conformational changes and possible interactions with its molecular environment. These cases produce two consequences. The first one, the energy of S_1' is almost dissipated, accommodating singlet excited state (S_1) from fluorescence emission originates. The second consequence is that not all the excited molecules not return to the ground stage by fluorescence emission.

Third stage: Fluorescence Emission.

Fluorescence starts when the fluorophore returns to its ground state S_0, by the emission of light of energy hv_{EM}. Due to the previous stage where the fluorophore achieve a relaxation stage, energy dissipation occurs so the energy of this photon is lower and has longer wavelength, than the excitation photon, hv_{EX}. Stokes shift is known as the difference in energy or wavelength by ($hv_{EX} - hv_{EM}$). This is essential for the sensitivity of fluorescence techniques since the emited light can be detected against a low background.

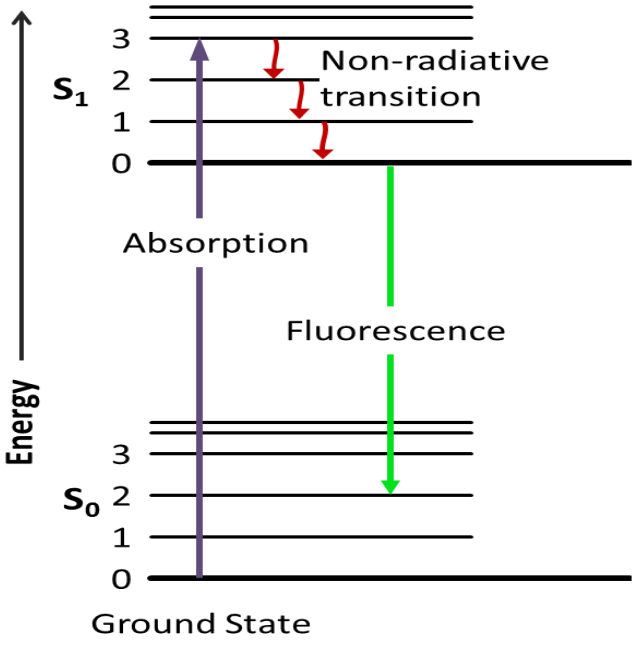

Figure 14. Jablonski diagram. [16]

The process of fluorescence is cyclic, since the same fluorophore can be excited and detected repeatedly unless destroyed. For fluorescence techniques, it is very important to know the fact that one fluorophore can generate hundreds of detectable

photons because it is essential to obtain high sensitivity of fluorescence. The discrete electronic transitions represented by hv_{EX} and hv_{EM} in spectrum. The bandwidths of the spectra, have to be taking into account for applications, because in some of them different fluorophores are simultaneously detected. [18]

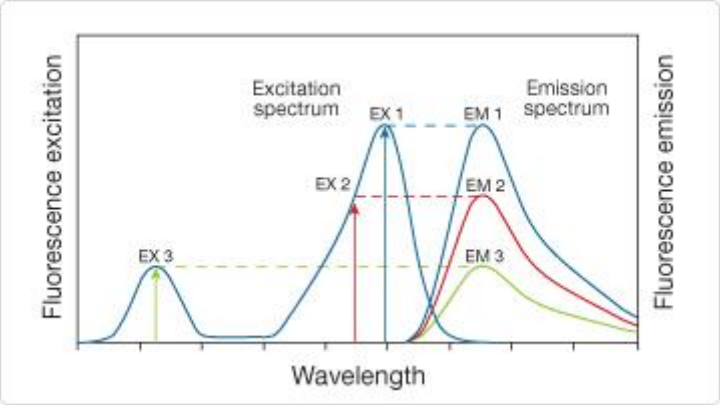

Figure 15. Fluorescence Spectra. [18]

The fluorescence emission intensity corresponds to the amplitude of the excitation spectrum; so the excitation at different wavelength of the same fluorophore is going to produce variations in the intensity but not change the emission profile. [18]

When using fluorescence as information source using a microscope or other imaging techniques which may be fitted with fluorescence such as endoscopy in this case. There are two options for fluorescence: the first is that the sample contains endogenous molecules that can fluoresce, so autofluorescence is detected; or in the case of not having these molecules, use specific molecules, fluorochromes, that have to be added to the sample in order to get fluorescence. [19]

The fluorochromes are chemical compounds that can emit light upon light excitation. [19]

Figure 16. Fluorochromes [20]

Fluorochromes can be divided in three groups: [19]

The first group consist on fluorochromes that need other molecules such as antibodies or lectines to bind to specific targets.

The second group are the fluorochromes that have inherent binding capacities. Other fluorochromes that are included in this group are fluorochromes that change their fluorescence properties when bound to different amounts of molecules.

The third group consists on fluorescent proteins that organisms can produced by themselves.

All these fluorochromes have different spectra properties, so they can be combined for multicolor specimen analysis.

Fluorescence microscopy needs some components in order to work properly to detect fluorescence. Light source to excite fluorescence in the specific spectra of each fluorochrome; it ultraviolet light and lasers are also used. In most of the cases filters have to be used since they allow the passage of light of a given wavelength, the range and the color needed to excite the fluorophore and block unwanted lengths. Objectives are also required to transmit light and provide high quality images. [20]

In medicine, some of the applications of fluorescence are characterization and identification of molecules in cells and tissues, immunological studies and so on. [20]

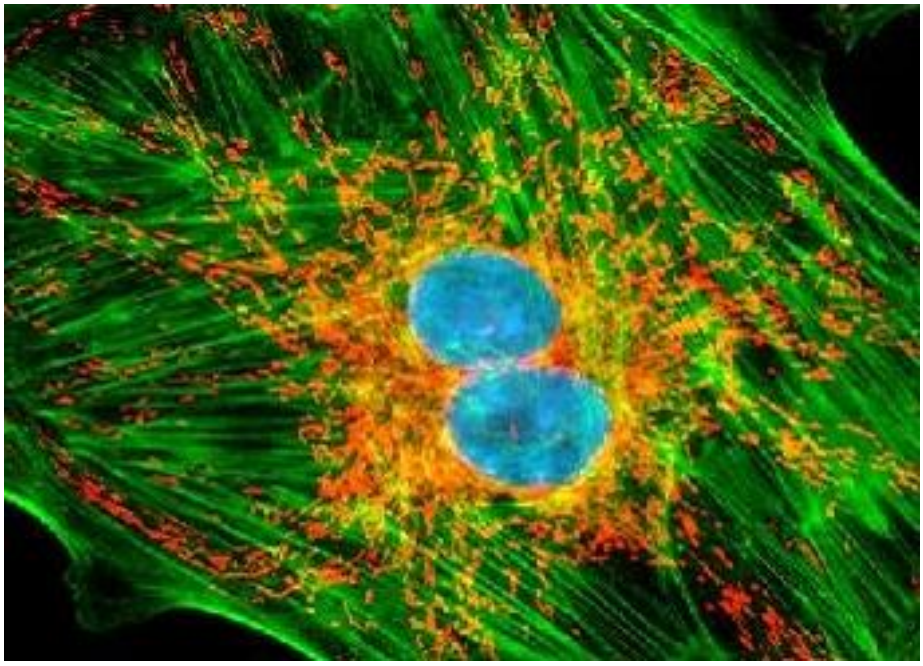

Figure 17. Gene expression by the using of specific fluorochromes. [21]

NIR fluorescence probes are molecules that are injected to the sample in order to see biochemical activity of cells to diagnose disease. This kind of probes gives advantages of high photon light penetration, reduce light scattering. So they are used for monitoring of disease progression in animal models, mapping of molecules events. NIR fluorescence probes are molecules that binds with biological molecules of the sample forming a complex. This complex can be excited through biological tissue,

because biological molecules are molecules that express low absorption for light in a spectral window between 680 nm and 900 nm. This is the reason why infrared excitation is used instead of visible or ultraviolet waves because these regions will produce interferences with biological molecules that have absorption characteristics. [22]

Blood has two different types of hemoglobin: oxyhemoglobin and deoxyhemoglobin. The difference between them is that hemoglobin is bound to oxygen while deoxyhemoglobin is not. The spectra of these molecules are slightly difference. *Figure 18*.

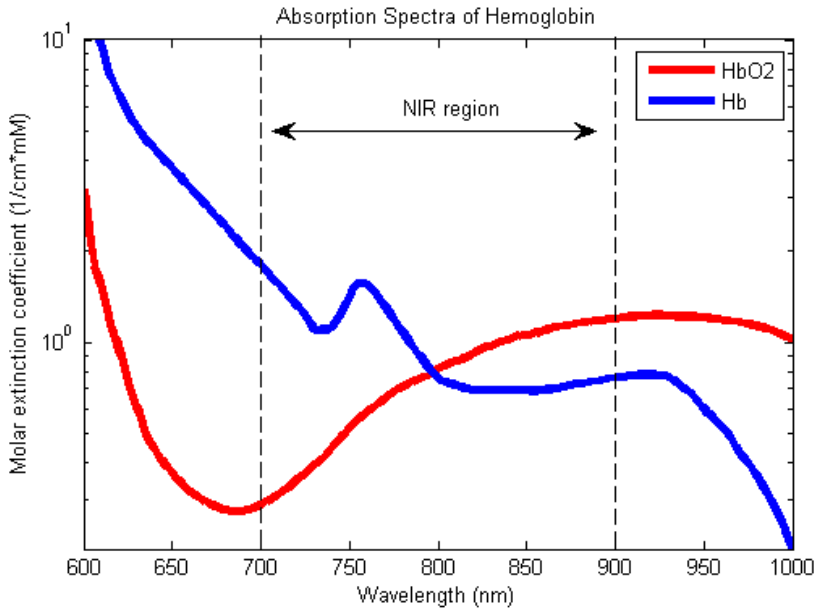

Figure 18. Hb and HbO spectrum [22]

Differences in Hb and HbO spectra allows measurement of relative changes in hemoglobin concentration through the use of light attenuation at multiple wavelengths. [22]

2. Motivation and Objectives

2.1 Motivation

Nowadays, multimodality techniques are becoming more and more important because of the need to combine anatomical and functional information. Until now, the way of combine these approaches has been made from the combination of images taken from different modalities but with some disadvantages related with differences in acquisition time, and difference in the position of the patient by involuntary movements or wrong calibration that can occur. This is a disadvantage when trying to combine the images as problems can be found, related to the orientation and position of the patient, whether involuntary movements produced by the patient´s breathing; or either by a deformation of the studied organ. Given these drawbacks, creating devices that are capable of simultaneous images from different modalities, can be major breakthrough for both the study and diagnosis of biology or diseases.

In medical imaging, endoscopy is a modality that allows the visualization of cavities, and has been limited to the field of visible light and has rarely been combined with other modalities except in surgery where endoscopy is sometimes used as a guide imaging technique. However, the possibility of including in an endoscope the fluorescence modality is a breakthrough and a great advantage in improving both detection and diagnosis of abnormalities due to the large information that fluorescence provides, such as visualization of autofluorescence substances as vitamin A; or visualization of substances labeled with fluorophores.

But besides multimodality, improving both accuracy and acquisition time among other factors, will help modernize the devices, in addition to increasing the quality of the images.

Once images are obtained, a suitable processing can be needed in most of the cases since this can greatly improve image quality. In recent years, three-dimensional representation of the data or obtained images, in medicine has expanded

considerably. 3D visualization is used for many fields of medicine and surgery, orthopedics, liver studies, tissue analysis, reconstruction of blood vessels, venous thrombosis, ischemia, etc. The information can be obtained from different kinds of studies whether it is ultrasound, computerized axial tomography, nuclear magnetic resonance, X-ray, endoscopy of which anatomical and functional information of the studied region or organ may be retrieved.

The possibility of combining all these factors: multimodality, greater precision and 3D reconstruction notably increases both quality of the instrument as the images that are obtained from it. Therefore research and development of new or improved technologies are the keys to advancing medicine. [13]

2.2 Main Objectives of this Work

Considering all the motivations of this project, the main objectives of this work are: in the first place, design the platform on which the sample is mounted; secondly image acquisition using a motorized control, allowing precise control of the entry of the endoscope in the sample; and finally image processing of obtained images for their 3D-reconstruction. Overall, the aim of this project consists on an appropriate control image acquisition and further processing, in order to be able to use this device for in vivo studies with small animals.

3. Materials & Methods

3.1 Endoscope

This project has used a commercial KARL STORZ ® endoscope, ***Figure 19***. This company is responsible for manufacturing and distributing endoscopes, medical instruments and devices.

In this case an endoscope was used belonging to the catalog Mainz COLOVIEW® System, a set for colonoscopic examination and biopsy in mice and rats. The endoscope model used is HOPKINS® Straight Forward Telescope 0^{O}, diameter 1.9 mm, length 10 cm, autoclavable, fiber optic light transmission incorporated, with color code green. Furthermore, this set contains a protection and examination sheath that 7 French catheter scale: 2.33 mm diameter ***Figure 20***, working length 8.5 cm, and 2 LUER-Lock cones, color code: blue. Another examination sheath, 9 Fr. ***Figure 20***, working length 7 cm, and working channel 3 Fr. ***Figure 20***, color code: red. And biopsy forceps, double action jaws, flexible, 3 Fr. ***Figure 20***, length 28 cm. [23]

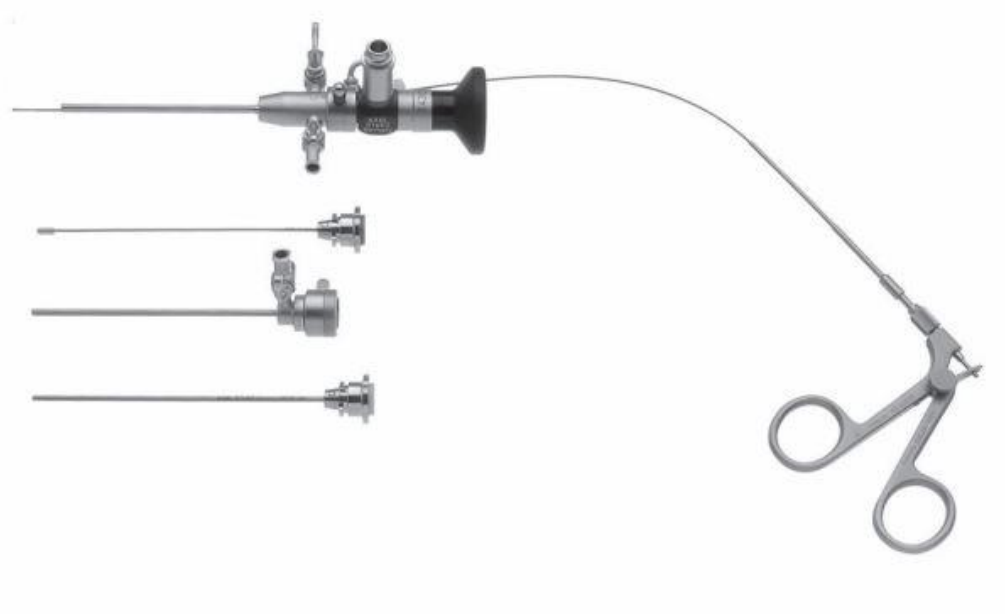

Figure 19. Endoscope used in this project [23]

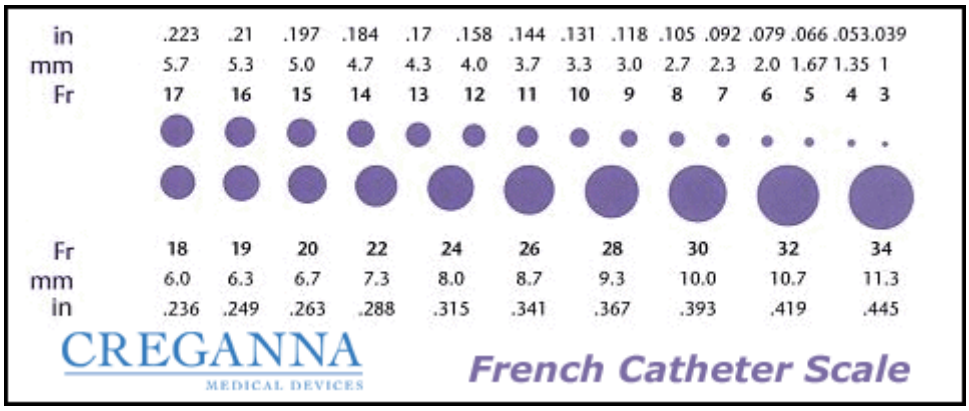

Figure 20. French catheter scale [24]

The endoscope is connected to two cameras of Allied Vision®, ***Figure 21***, which offers a wide range of cameras for science and research. Allied Vision® makes cameras able to work perfectly with microscope, telescopes or other scientific equipment for the possible observation of small details. The camera models are Allied-Vision® Manta G-145C IRC and Allied Vision® Manta G-145B NIR. Both cameras have the same appearance but with slight differences in the characteristics and properties. The first camera is a low cost GigE Vision camera with a Sony ICX445 sensor. It runs at 30 fps (frames per second) (full resolution). This camera incorporates three LUTs, sophisticated color correction algorithms, a robust metal housing, and many modular options like angled heads or board level versions. And the second one is an NIR optimized camera with Sony ICX285 ExView HAD sensor, that is CCD Progressive. It provides enhanced NIR sensitivity, and has an excellent anti-blooming, with a resolution of 1388x1038; and with a cell size of 6.45µm. [25]

Figure 21. Allied Vision camera [25]

Fluorescence imaging requires filters with specific spectral and physical characteristics. Depending on the type of microscope and the applications, different characteristics are needed in a filter to perform the desired application. [26]

The filters chosen are:

First set	Second set	
Short pass 800 nm	Low-pass 700 nm	Excitation Filter
800 nm	700 nm	Dichroic Beamsplitter
825-835 nm	725-735 nm	Emission Filter

Table 2. Chosen filters

These set of filters are known as filter block composed by an excitation filter, emission filter and dichroic beamsplitter. ***Figure 22***

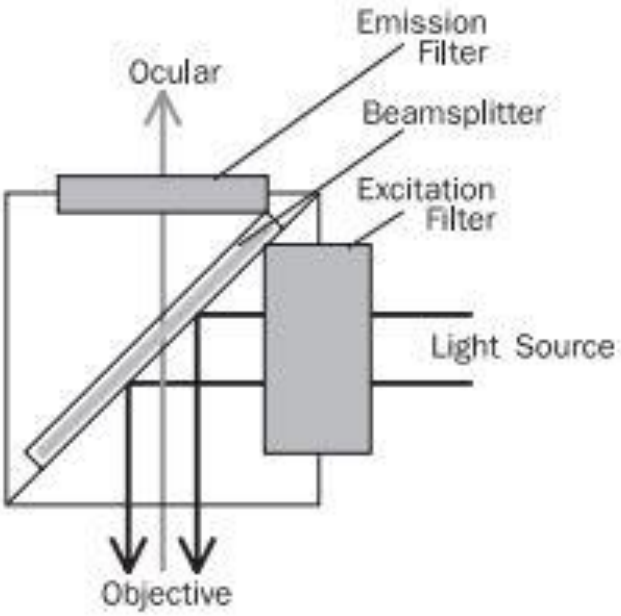

Figure 22. Set of filters [18]

The excitation filter is the responsible for transmitting only light with wavelengths capable of exciting the specific dye. The one of the excitation filters that have been chosen are short pass filters. This kind of filters are designed to have sharp transition from transmission to reflection making them useful for fluorescence applications. The second excitation filter is a low-pass filter. This filter is used to stop high frequencies and reduce the effects of moiré and false color caused by high frequency waves. [26]

The emission filter is used for attenuate all light transmitted by the excitation filter and for transmit any fluorescence emitted by the sample.[26]

The dichroic beamsplitter is a coated glass set at 45 degree angle to the optical path of the microscope. This mirror only reflects one color, the excitation light, but transmits another one, the emitted fluorescence.[26]

In this project the fluorochromes used are Cy5.5 and Cy7.5.

Cy5.5, *Figure 23*, is an ester that are is used for the labeling of amino-groups in peptides, proteins, and oligonucleotides. [27]

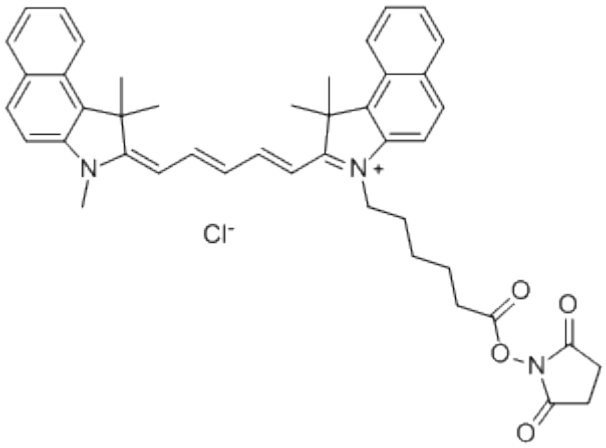

Figure 23. Cy5.5 molecule [27]

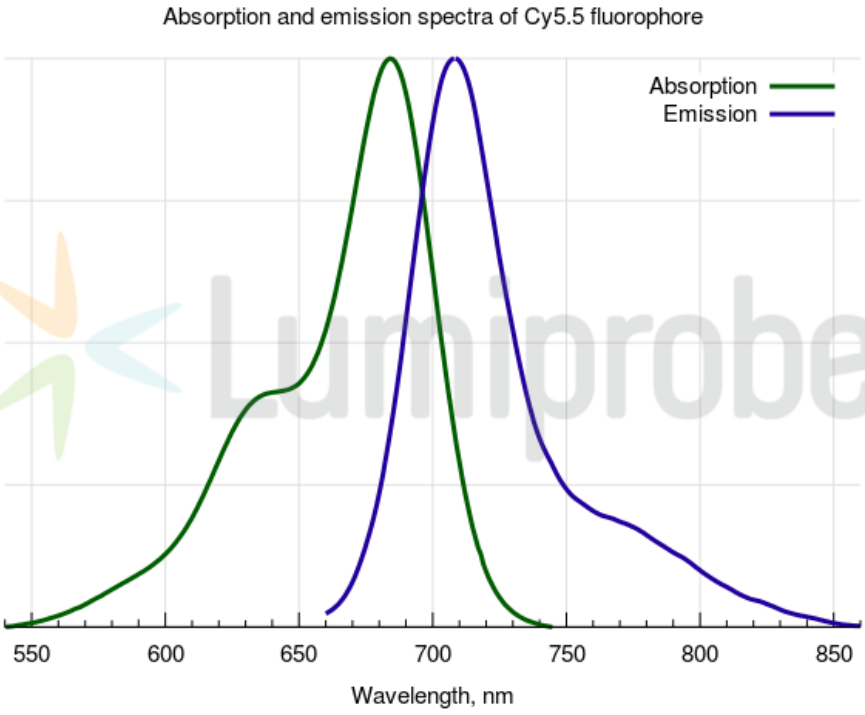

Figure 24. Absorption and emission spectra of Cy5.5 [27]

Cy7.5, *Figure 25*, is also used for labeling biological molecules containing amine groups by in vivo imaging, because cyanine7.5 is a NIR dye with long-wave emission. [28]

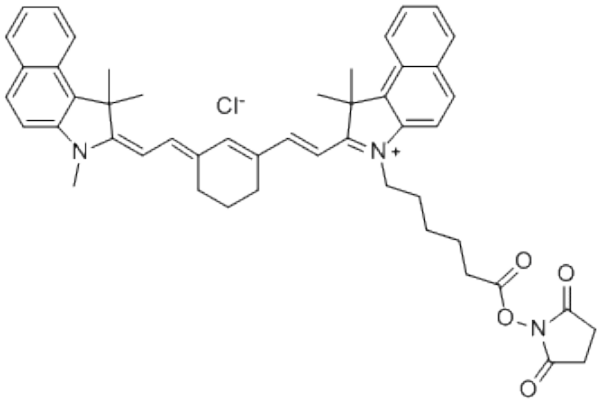

Figure 25. Cy7.5 molecule [28]

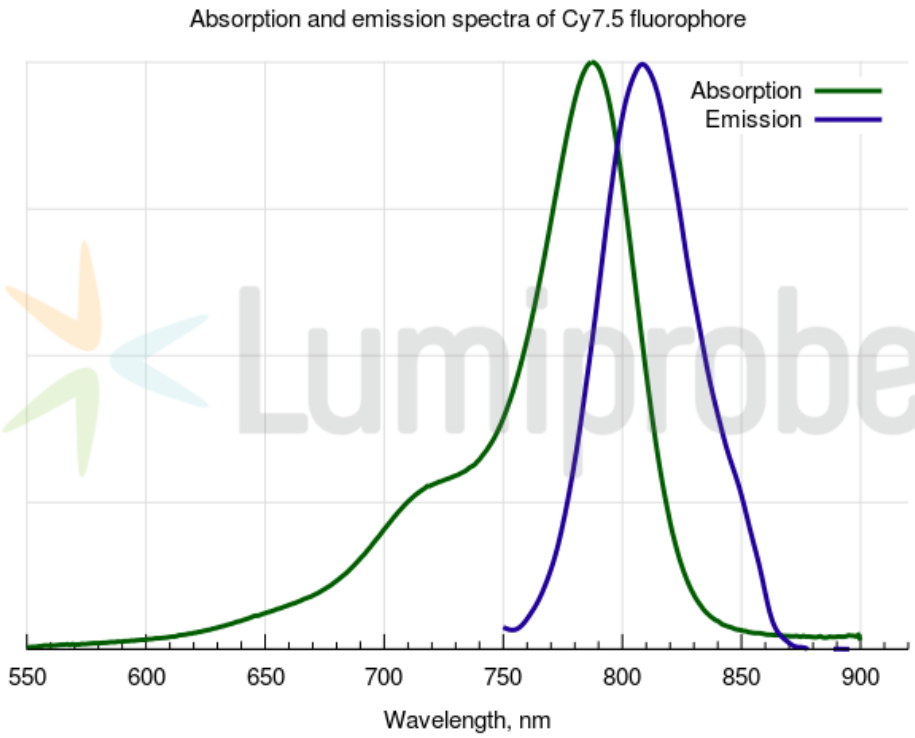

Figure 26. Absorption and emission spectra of Cy7.5. [28]

The final result of the combination of all these components are include in *Figure 27*.

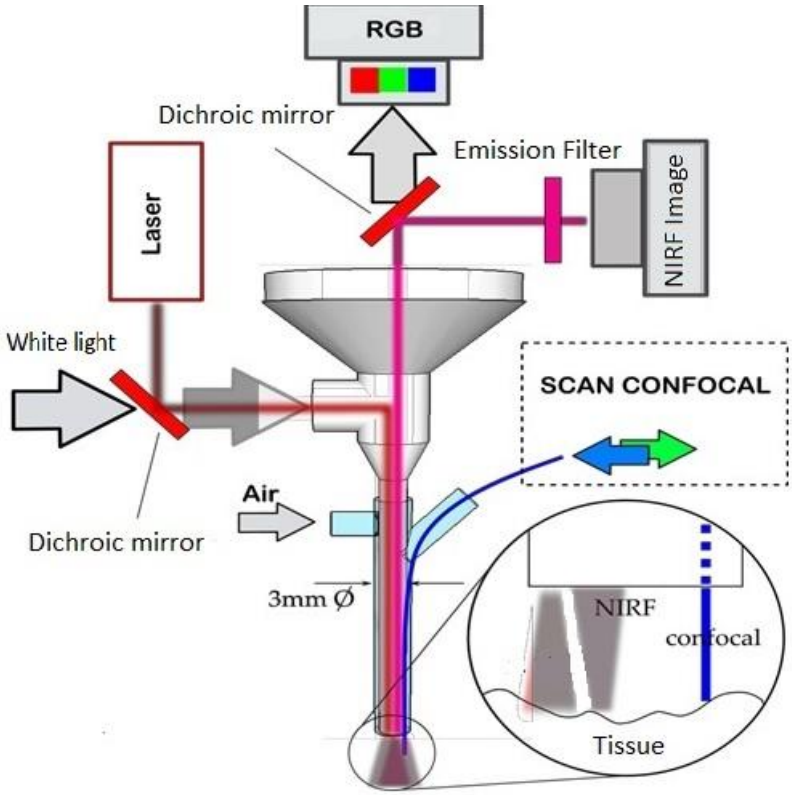

Figure 27. Scheme of the endoscope with the cameras.

3.2 Image acquisition

In this section, materials and methods used to acquire images, are going to be explained. Starting with the materials for the design of the bed where the sample is mounted, followed by the methods used to control it, making use of a motor. And finally the materials and methods for analyzing obtained images.

3.2.1 Design of the Bed

To preserve the stability and integrity of the motor it is necessary to avoid direct contact between the motor and the sample, therefore a custom made piece was attached to the motor stage. This piece is bolted to the motor to prevent any

movement of the piece when the images are taken. The piece has the same dimensions as the movable part of the motor so that the piece does not protrude from the motor. Both the width and the length are sufficient to accommodate the sample, either for in vivo studies of small animals or a sample ex vivo that can be mouse intestine.

The material used is a transparent plastic polymer, and was also made by the department of mechanics of the University Carlos III of Madrid. Since they used patchwork pieces the manufacturing has had no cost for the project.

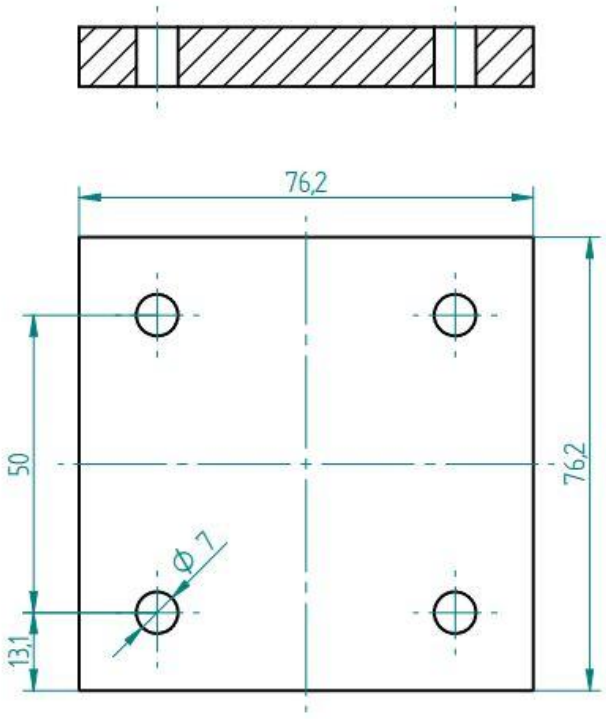

Figure 28. Planes of the piece.

Figure 29. Real piece.

3.2.2 Control of the Motor

Figure 30. Zaber´s T-LS motorized linear translation stage [29]

For proper operation of the motorization of the bed, Zaber´s T-LS motorized linear translation stage, *Figure 30*, has used, that is computer controlled. This kind of

device has 0.1µm resolution, and has 28 mm travel. In the next table , the features of the device used are exposed. [29]

Model	T-LS28M-S
Travel Range (mm)	28
Microstep Size (Resolution (µm))	0.099
Accuracy (µm)	29
Repeatability (µm)	< 2
Backlash (µm)	< 14
Minimum Speed (µm/s)	0.93
Maximum Speed (mm/s)	6.5
Maximum Centred Load (N)	100
Maximum Cantilever Load (N*cm)	125
Weight (Kg)	0.59

Table 3. Features of the motor [29]

The device can be connected to the RS-232 port or USB port of any computer. The company provide free software to control easily the Zaber device by selecting the device that will be moved, selecting a command and enter the desired position. This software is Zaber Console. [29]

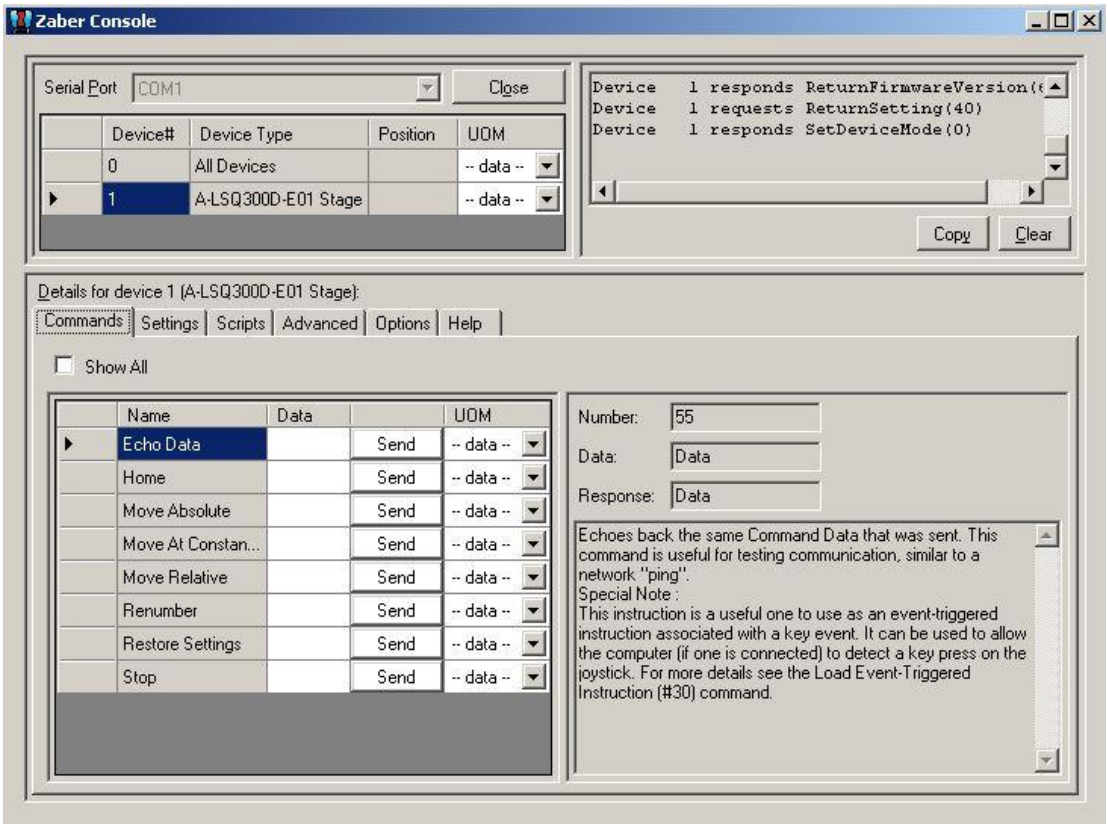

Figure 31. Zaber Console

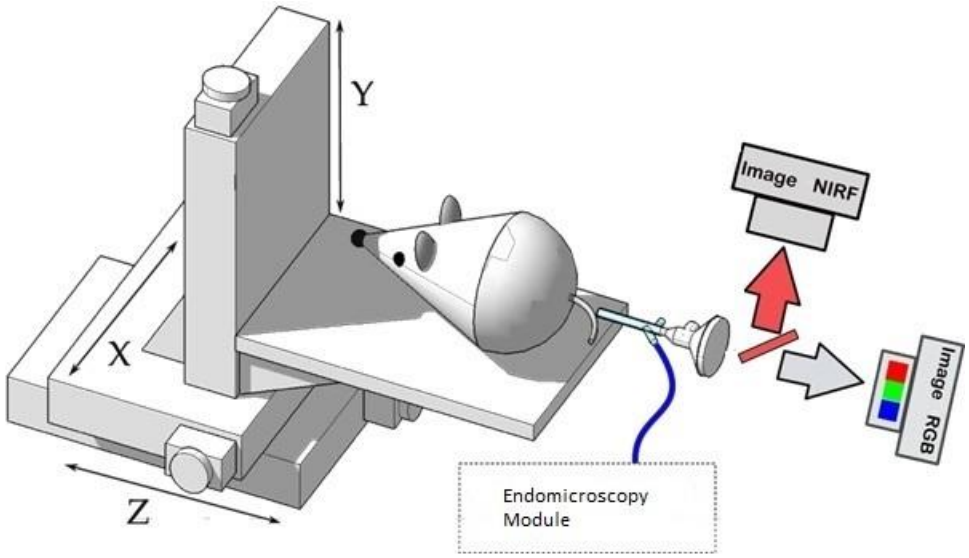

Figure 32. Motorized bed.

In addition to its own software, this type of device offers a possible control by other software included in LabView, Matlab, C, Phyton, Visual C++, plus some others.

In this case, it has been decided to use the LabView software for controlling and managing the device. "The LabView instrument driver" is used, that includes subVIs and type definition that make it easy to start controlling Zaber devices with LabView. [30]

To obtain the driver must be downloaded from the internet, it can be found on the website: http://www.zaber.com/wiki/Software/Binary_Labview_Driver.

Development of the program.

First, it is necessary to specify the instrument that is going to be used, in this case the Zaber device. Using the VISA command, the program allows the user to identify the instrument that needs in each moment. Once the device is selected, the initialize VI is called. This command is called only once at the beginning of the program. Its function is simple return the motor to its starting position (0 mm), so the motor stars its movement at the same position and the record of the images start at this position too. In addition, this command permits the full range of movement since this motor can only move a distance of 28 mm. [31]

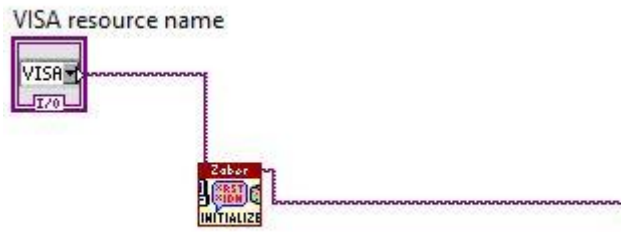

Figure 33. Commands for specify the instrument and initialize the motor.

The rest of the code is content in a while-loop in order to take images continuously. For control the movement of the motor, in the main program is included a subVI. In this subVI is necessary to specify the instrument again. Since the unit of the motor is in microstep size units, a converter is used to change this units to mm, in order to make more easy interface with the user. [31]

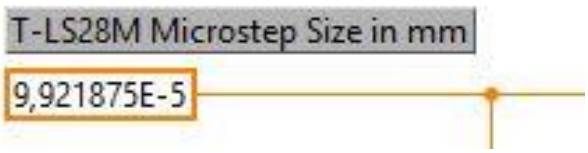

Figure 34. Unit converter

The next step is the instructions of move absolute and set speed, to introduce the speed at which the motor is going to move, and the distance that the motor will travel. To introduce these instructions write command VI is used. This command write the instruction to a Zaber device through the serial port. It is used twice to include both instructions. Write command is followed by read command VI whose function is read a response packet from the serial port. [31]

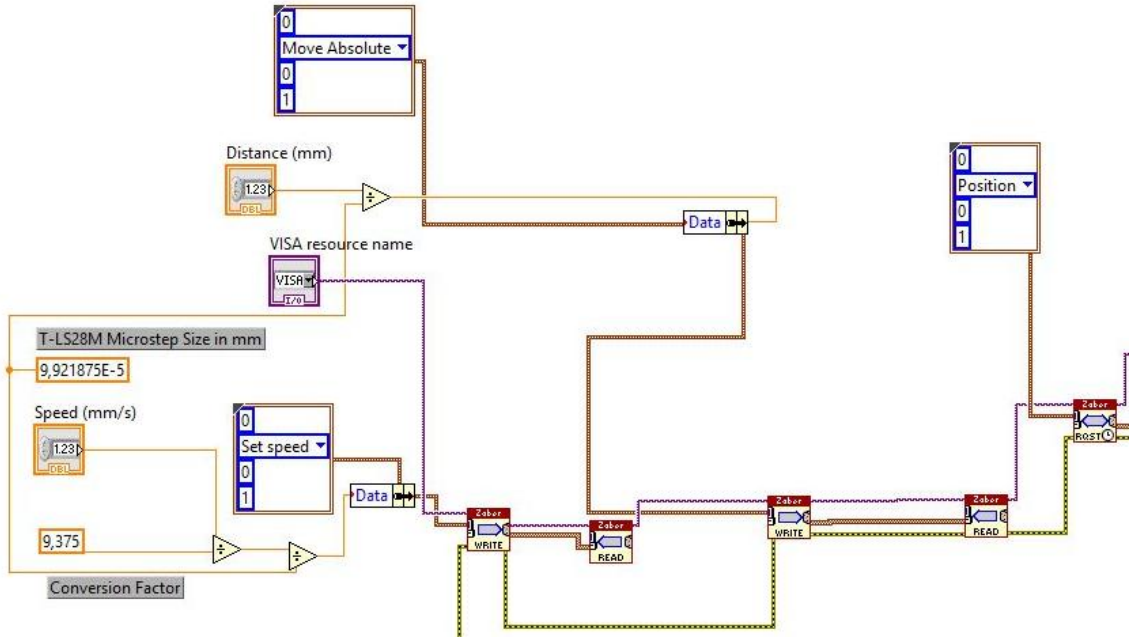

Figure 35. Code to send and read the commands to the motor.

The motor position is a constant that is required, so to get that constant, the instruction of position is used followed by request command VI. This command sends a instruction and waits for a response. If the response is an error, sets an error code, that the reason why error in and error out commands are used along the program. [31]

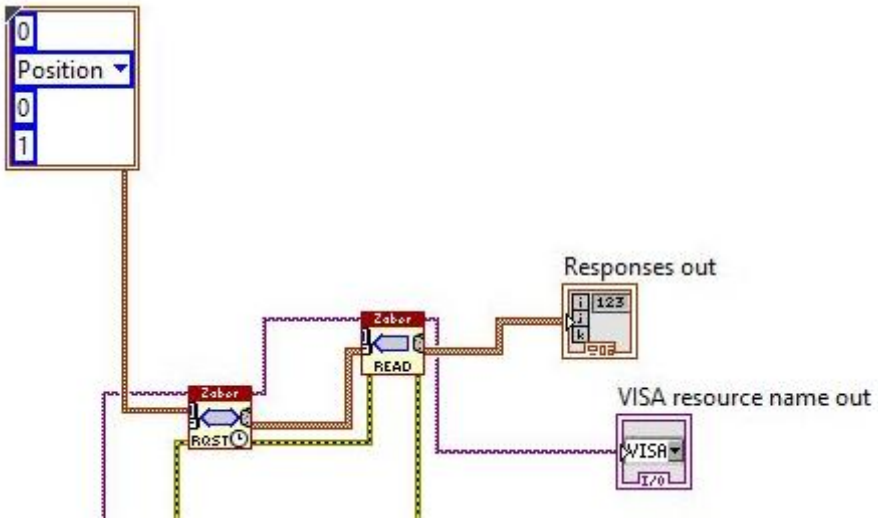

Figure 36. Request command VI

Once the program of the motor´s control is complete, the following step is to save the images with the position where the images are taken, as the name of the images. The position is a constant that is extracted from the subVI that controls the distance and the speed of the motor, explained above. That data is introduced in a loop to get the position each time images are taken. In addition, the position is transformed to a number.

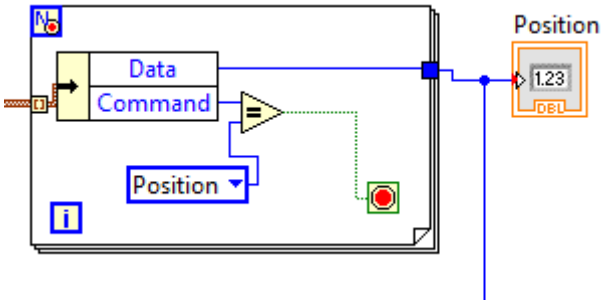

Figure 37. Position constant in number format.

These constant numbers are transformed one by one into a string by number to decimal string command.

Figure 38. Number to string command

These strings are inserted into the subVI called save_images and also images that are taking, are introduced. Within this subVI, IMAQ create command, that creates a temporary memory location for an image. And it is needed to indicate the type of image RGB(U32). The result of this command, image Dst, is introduced into another command IMAQ copy that needs also image Src, that are the obtained images. This function is used to copy the specifications and pixels of one image into another image of the same type. Then, the images have to be stored in a folder that is previously decided and in wanted format .tiff.

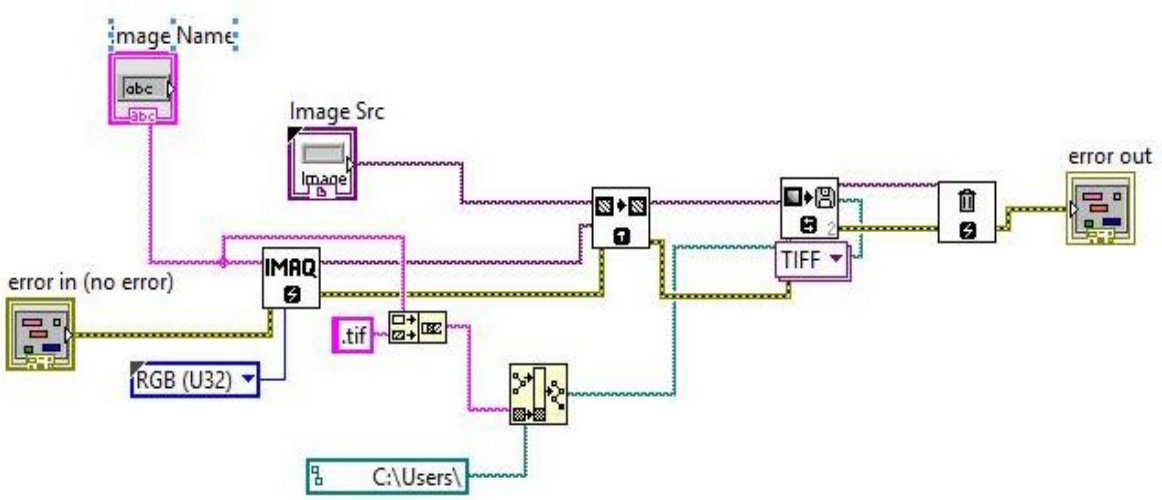

Figure 39. Save_images program

The user interface is shown in *Figure 40*.

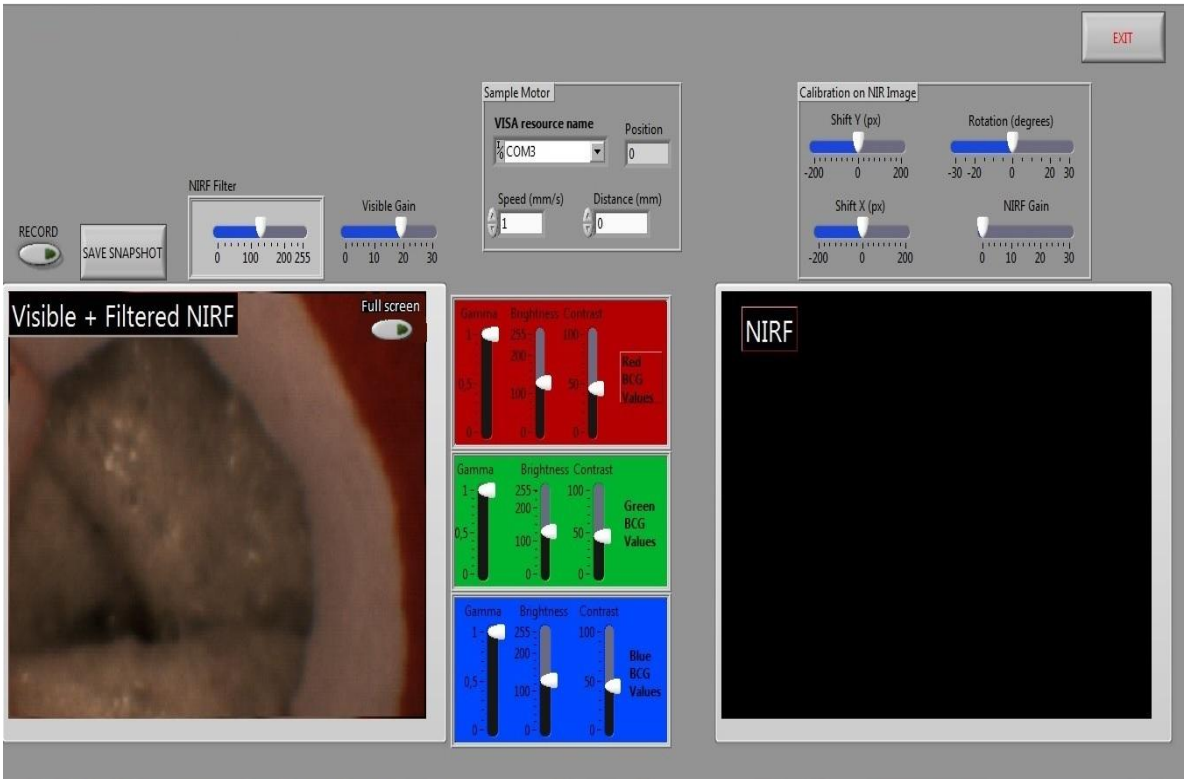

Figure 40. User interface

3.3 Image analysis

The images used in this analysis were obtained from ex vivo study of murine´s colon. *Figure 40*

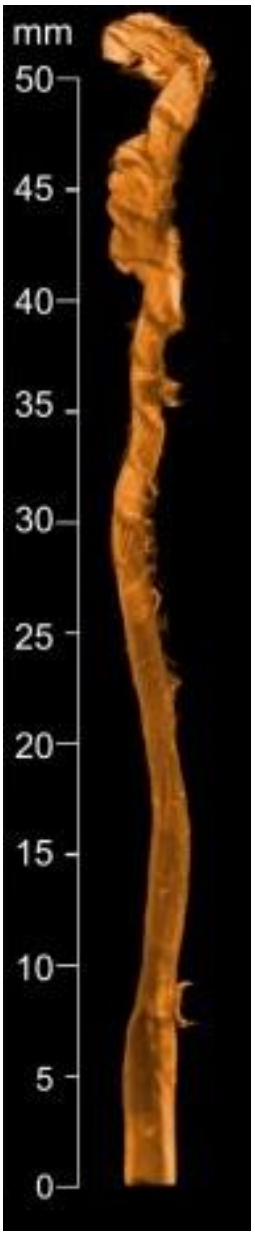

Figure 41. Murine´s colon

Once the images have been taken, saved with the name of the position where they were taken, and stored in the folder that has been decided, these images are to be processed to get 3D information about them. For them, it was decided to use

Matlab software, because it is a mathematical tool that has toolboxes for image processing.

First, the content of the folder that contains the images, is shown. This allows the access to all the images by using the 'dir' matlab function. The next step is to determine the number of images in the folder based on the length matlab function. As analyze each image is needed, a for loop is required to run all images in the folder.

As the name of each image reveals the position to which they were taken, this data is extracted, by using read_file(k).name, since the images are stored in form of structure. . Then, the images are converted to grayscale by eliminating the hue and saturation information while retaining the luminance. the noise of the images must be remove so a 2-D median filter is used . The next is to get the background and for that the histogram is calculated so the background will correspond to the first value.

It is necessary to know that each images have a position so to analyze the depth is necessary to calculate the center and radius covering most of the image, because to get 3D information the idea is to use farthest part of the image from the center. Once the center and the radius are calculated, the program shows a image which contains the original image and the circle formed with the radius and the center previously calculated, *Figure 41.*

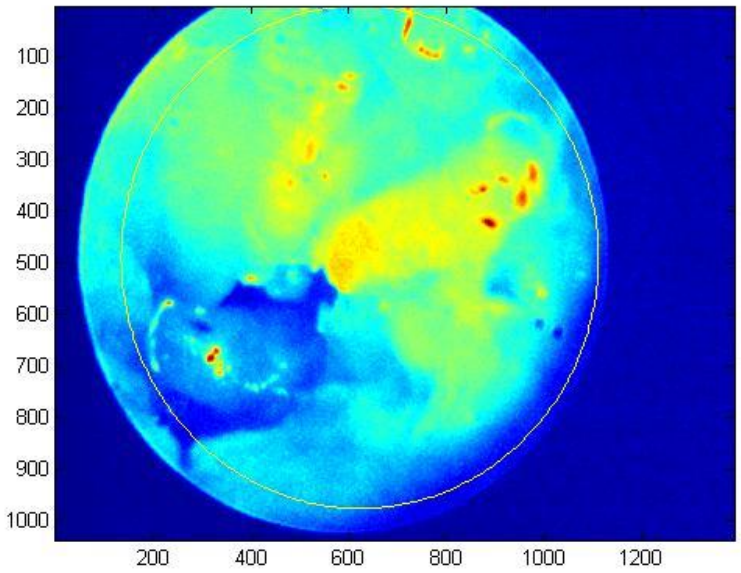

Figure 42. Center and radio defined

From this image, the radius and center can be modified in order to ensure that the circle includes only the image and not part of the background and also ensure that the circle is not too small.

When the circle is locate at the most appropriate site, it is analyzed. This analysis, instead of using interpolation, it is used a simpler code that consists on compute the first neighbor.

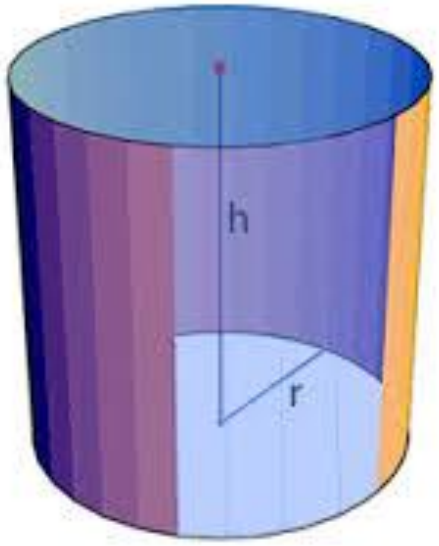

Figure 43. 3D cylinder

The most simple way to get 3D information from a cylinder is changing the Z position, in Figure 43, the Z position is the height. In this case, the radius is also reduced since taking small radius the data are more far from the endoscope.

4. Results and Discussion

4.1 Endoscope images

The following images that were obtained from a murine colon.

Endoscopic images have a characteristic appearance, they are circular, figures below (*42, 43 and 44*). This feature is due to the lens used. This lens is used in order to observe as much as possible.

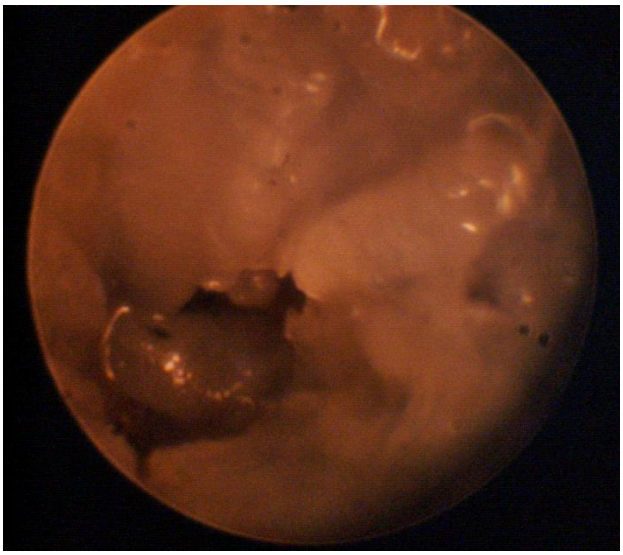

Figure 44. Endoscopic image

Fluorescence images could not be included since fluorophores have come too late to include them herein.

4.2 3D- Reconstruction

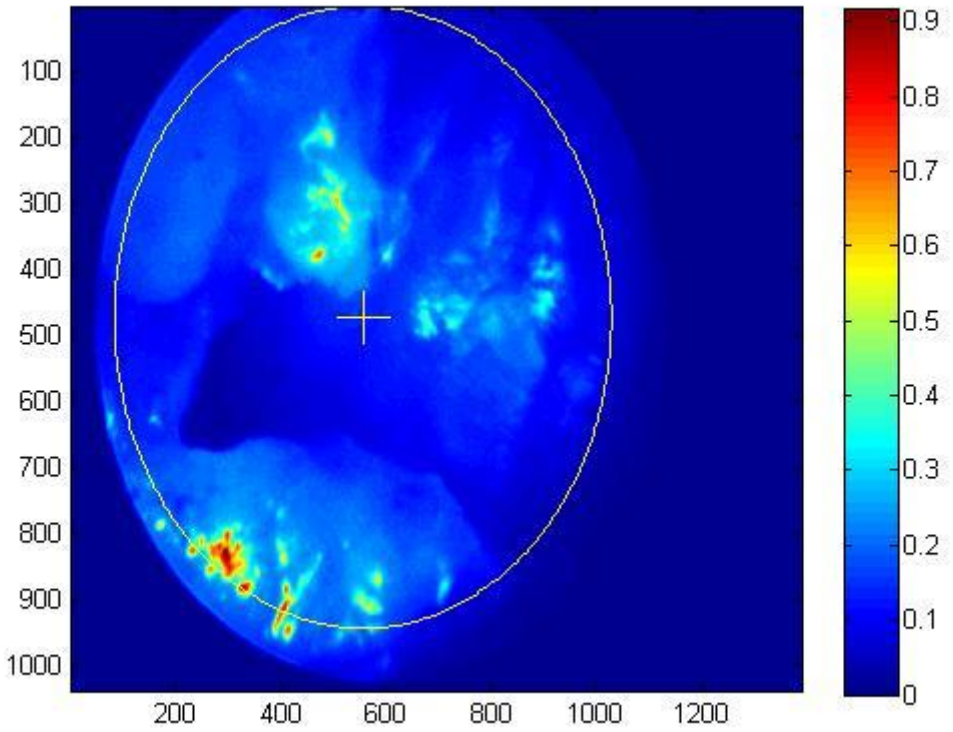

Figure 45. Endoscopic image with the circle of analysis

In this image, *Figure 45*, it can see the endoscopic images and the obtained circle from the center and radius that have been calculated. This radius is the first radius that is analyze, so the image analyzed is the image inside the circle. Taking this radius as a reference, the program takes smaller radii for the analysis of the depth. Each radius is analyzed.

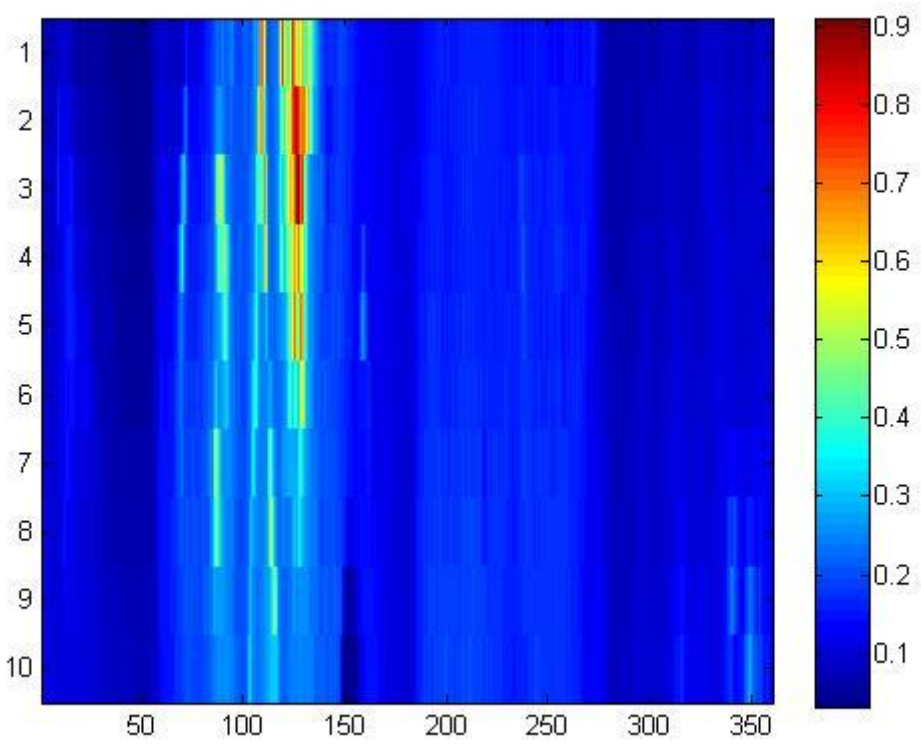

Figure 46. Graph Z position vs. angles

In the original image there is a position. In each Z position there are two axis, X and Y. The pixels of the image represent on position in the space that is unknown. In order to get 3D information, it is analyzed the part of the image that is far from the center.

With a chosen radius, its coordinates are transformed to a graph that represents in 2D of the intensity and the angle. In order to obtain a 3D image the radius can be maintained and changes the Z position. But changing the radius, 3D information can be obtained since small radius means information that is far from the endoscope.

So taking this into account, *Figure 46*, a 2D intensity vs angle. This graph represents the values of the pixels that are contain at a certain Z position and radius. So it expresses the changes in the radius and Z position so the result is changes in angles.

5. Conclusions & Future Works

In this project, the system made allows the motor control of the introduction of the endoscope into the sample with an accuracy around microns. In addition, this system can store the obtained images and know the position at the images were taken. This supposes a great advantage because it admits the comparison of two images of the same place taken at different time.

It is noteworthy that images can be taken with visible light or infrared light to produce fluorescence. This kind of images give information about many biological processes.

Further analysis of the images is also included in this project to obtain 3D information of the images. 3D information allows make more exact accurate diagnoses, plan surgeries with greater certainty and comfort, and so on.

One the possible improvements or future work that can be made is improving the reconstruction system, trying to represent the information in a more visual form by projecting the images into a cylinder.

To finalize, it is essential to note that the purpose of this endoscope is animal models that allow more detailed studies that gives more precise information for a better understanding of biological processes and diseases.

6. Budget

To finish the project, it has been included a section about the budget, so it has been made an actual calculation that has led the project.

This budget is for a company that trying to make this project. For academic research some of these prices are considerably reduced. For example the prices of the software elements differ from industry to academic research since the price of Matlab in academic research is about 600€ and LabView for project is free.

6.1 Cost of the materials

Quantity	Description	Price per unit	Total price
1	Optica HOPKINS®	2.088,00€	2.088,00 €
1	Protection & Examination Sheath	413,00€	413,00€
1	Examination Sheath	404,00€	404,00€
1	Biopsy Forceps	449,00€	449,00€
	Total price		3.354,00€

Table 4. Price colonoscopic examination and biopsy

Quantity	Description	Price per unit	Total price
1	Motorized Linear Stage, 28 mm travel, M6 thread, RS-232 plus manual control	1,060.96.€	1,060.96.€
	Total price		1,060.96€

Table 5. Price of Zaber´s motor

Quantity	Description	Price per unit	Total prices
1	Allied-Vision® Manta G-145C IRC	2,395.00€	2,395.00€
1	Allied Vision® Manta G-145B NIR.	2,595.00€	2,595.00€
	Total price		4,990.00€

Table 6. Price of the cameras

Quantity	Description	Precie per unit	Total price
1	Ø1" Laser Line Filter, CWL = 730 ± 2 nm, FWHM = 10 ± 2 nm	82,93€	82,93€
1	Ø1" Laser Line Filter, CWL = 830 ± 2 nm, FWHM = 10 ± 2 nm	82,93€	82,93€
	Total price		165,86€

Table 7. Price of the filters

Some optometric materials were required for the construction of the whole system, are price to 1,057.74€

6.2 Personal cost

Quantity	Description	Price/hour	Total hours	Total price
1	Engineer Junior	25€	400 hours	10,000€
1	Engineer Senior	35€	600 hours	21,000€
	Total price			31,000€

Table 8. Personal price

6.3 Software cost elements

Quantity	Software	Total price
1	LabView	1,288.90€
1	Matlab	6,000€
Total price		7,288.90€

Table 9. Softwares price

6.4 Total price

Total Price of the project	43,932.45€

Table 10. Total price

8. References

[1] (Harrowgastro)

< http://www.harrowgastro.co.uk/endoscopy.html>

[2] (NHS choices Your health, your choices, 2014)

<http://www.nhs.uk/conditions/Endoscopy/pages/introduction.aspx>

[3] (Gov.scot, 2006)

<http://www.gov.scot/Publications/2006/03/03155226/2>

[4] (npecomp)

<http://www.nppecomp.ru/eng/dok/h_end.htm>

[5] (Wikipedia, 2015)

<https://en.wikipedia.org/wiki/Borescope>

[6] (Wikipedia, 2015)

<https://en.wikipedia.org/wiki/Fiberscope>

[7] (Cancer.Net, 2014)

<http://www.cancer.net/navigating-cancer-care/diagnosing-cancer/tests-and-procedures/types-endoscopy>

[8] (Olympus-me)

<http://www.olympus-me.com/medical/en/medical_systems/applications/pulmonology/diagnostic_bronchoscopy/bronchial_biopsies__tbb__and_lung_biopsies__tblb_/bronchial_biopsies__tbb__and_lung_biopsies__tblb_.html>

[9] (Cancer.org)

<http://www.cancer.org/treatment/understandingyourdiagnosis/examsandtestdescriptions/endoscopy/endoscopy-new-types-endoscopy>

[10] (Mayoclinichealthsystem.org)

<http://mayoclinichealthsystem.org/locations/eau-claire/medical-services/gastroenterology-and-hepatology/capsule-endoscopy>

[11] (Webmd)

<http://www.webmd.com/virtual-colonoscopy-topic-overview?page=2>

[12] (Medicimaging)

<http://www.medicimaging.com/radiology.html>

[13] (In vivo wide-area cellular imaging by side-view endomicroscopy, 2009)

[14] (Zmpbmy.meduniwien)

<http://www.zmpbmt.meduniwien.ac.at/forschung/optical-imaging/morphological-imaging/endoscopic-oct/>

[15] (Europepmc)

<http://europepmc.org/abstract/med/16981113>

[16] (Wikipedia, 2015)

<https://en.wikipedia.org/wiki/Fluorescence>

[17] (Energyeducation)

<http://energyeducation.ca/encyclopedia/Energy>

[18] (Lifetechnologies)

<http://www.lifetechnologies.com/es/en/home/references/molecular-probes-the-handbook/introduction-to-fluorescence-techniques.html#head1>

[19](Imaging-Git)

<http://www.imaging-git.com/science/light-microscopy/fluorescence-basics-techniques-advantages>

[20] (3.unipv)

<http://www-3.unipv.it/webbio/anatcomp/freitas/2010-2011/Micr_fluor_busb2010.pdf>

[21] (Cicwebserver)

<http://cicwebserver.dep.usal.es/confocal/web/WEB/apartados%20web%20%20micro/aplicaciones%20confocal.dwt>

[22] (Wikipedia, 2015)

<https://en.wikipedia.org/wiki/Near-infrared_window_in_biological_tissue>

[23] (Karlstorz)

<https://www.karlstorz.com/cps/rde/xbcr/karlstorz_assets/ASSETS/2165810.pdf>

[24] (Wikipedia, 2015)

<https://en.wikipedia.org/wiki/French_catheter_scale>

[25] (Alledvision)

<https://www.alliedvision.com/en/products/cameras.html>

[26] (Depts.washington)

<http://depts.washington.edu/keck/handbook3.pdf>

[27] (Lumiprobe)

<http://www.lumiprobe.com/p/cy55-nhs-ester>

[28] (Lumiprobe)

<http://www.lumiprobe.com/p/cy75-nhs-ester>

[29] (Zaber)

<http://www.zaber.com/documents/ZaberCatalog.pdf>

[30] (Zaber)

<http://www.zaber.com/wiki/Software>

[31] (Zaber)

<http://www.zaber.com/wiki/Software/Binary_Labview_Driver>

ANNEX I

```
%Load Images
clear
clc

Read_file = dir('C:\Users\Lorena\Desktop\Images\*.tif'); %The format of the images
can be changed
Number_images = length(Read_file);

for k = 1:Number_images %Run all the images that the folder has.

    file=Read_file(k).name; %Obtain the number of the images
    n=strfind(file,'.');

   Name=('C:\Users\Lorena\Desktop\Images\');
  ImageRGB=imread(strcat(Name,file));

  I=rgb2gray(double(ImageRGB)/255);
  Imed = medfilt2(I,[5 5]); %Filter the image to reduce the noise as much as possible.

  [h,x]=hist(Imed(:),10);
  bckg = x(1);%Get the background.

  %Obtain an image without the background
  [Ny,Nx]=size(Imed);
  Ibin = Imed>bckg;

  %With this code we are obtaining the center and the radius
  x = (1:Nx)-Nx/2;
  y = (1:Ny)-Ny/2;
  [XX,YY] = meshgrid(x,y);

  Xcm = sum(sum((XX.*Ibin)))/sum(sum(Ibin)) + Nx/2;
  Ycm = sum(sum((YY.*Ibin)))/sum(sum(Ibin)) + Ny/2;

  Xcut = Ibin(floor(Xcm),:);
  idx = find(Xcut>0);
  Xmax = (max(idx));

  Ycut = Ibin(floor(Ycm),:);
  idy = find(Ycut>0);
  Ymax = (max(idy));

  Radx = Xcm - Xcut;
  Rady = Ycm - Ycut;
```

```
    Rad = min([Radx Rady]);

    figure(1)
    clf
    imagesc(x+Nx/2,y+Ny/2,Imed)
    hold on
    plot(Xcm,Ycm,'y+','MarkerSize',20)
    tita =linspace(0,2*pi,360);
    plot(Rad*cos(tita)+Xcm,Rad*sin(tita)+Ycm,'y-');

%Analysis of different radius.
    dRad = 10;
    for ir=1:10,

       pointsx = (Rad - ir*dRad)*cos(tita)+Xcm;
       pointsy = (Rad - ir*dRad)*sin(tita)+Ycm;

      for ip = 1:length(pointsx),
         % Find the first neighbour.
         if (pointsy(ip)>0) && (pointsy(ip)<=Ny) && (pointsx(ip)>0) && (pointsx(ip)<=Nx),
            Zpos(k, 1:(n-1)) = file(1:(n-1));
            Ival(ip,ir,k)= Imed(round(pointsy(ip)),round(pointsx(ip)));
            Angle = tita;
            Radius(ir) = Rad - ir*dRad;

         end
      end
    end
end
colorbar
figure
polar(tita,Ival(:,2,1)')
figure
imagesc(Ival(:,:,1)')
colorbar
```